Study the of the secretion flagellar proteins in the *rcsC11* mutant to serve as a model of vaccine against *Salmonella Typhimurium*

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**Background**

The members of genus *Salmonella* are Gram-negative bacteria causative of foodborne diseases in human and animals (1). *Salmonella* can survive and respond to adverse environmental changes through the regulation of gene expression mainly by the two component regulatory systems. The RcsCDB system controls a variety of cellular functions, mainly those genes involved in virulence (2-7).

The use of attenuated strains of *Salmonella* as vaccines is a useful method to transport heterologous antigens to eukaryotic cells. We are interested in the study of the role of RcsCDB system in the *S. Typhimurium* virulence responsible to produce gastroenteritis in human and typhoid fever in mice (8). Previously, we showed that the *rcsC11* mutant dramatically attenuates bacterial virulence and when inoculated into mice intraperitoneally led to an immunogenic response that resulted in a protection (9). This result allowed us to postulate that the RcsCDB system is a good candidate for vaccine development. On the other hand, the RcsCDB activation by the *rcsC11* mutant reduces the expression of the genes encoding invasion proteins as well as those involved in the flagellin synthesis (10,11). Here, we proposed that bypass of the flagella synthesis repression in the *rcsC11* mutant could be used to increase the host immune response without modifying the mutant virulence attenuation.

**Main Activities Conducted**

In order to bypass the inhibition of the *flhDC* expression in the *rcsC11* mutant, point mutations affecting the RcsB binding site were introduced into *flhDC* promoter region. The point mutations were chosen based upon an earlier mutagenesis study (Wozniak *et al.*, 2009). We used the following mutants: -190::A (BS#1), -189C:T (BS#4) and -198A:G (BS#5) (Wozniak *et al.*, 2009). The introduction of these point mutations in the *S. Typhimurium* 14028s strain and the isogenic *rcsC11* mutant was carried out by transduction with the phage of *S. Typhimurium* P22 HT105/1 int-201.

The resulted mutants were characterized for the effect on motility. The point mutations BS#1, BS#4 and BS#5 significantly increased motility compared with the wild-type strain. Surprisingly, the introduction of these mutations in an *rcsC11* strain restored the motility of this strain at wild-type levels. We selected the *rcsC11* BS#1 mutant for the next assays.

Transcriptional fusion to the *fliL* gene was utilized to investigate the effect of the BS#1 point mutation in the *rcsC11* mutant. We observed that the BS#1 mutation in the *rcsC11* mutant was able to bypass the inhibition of RcsB regulator on the *flhDC* transcription.

For the analysis of the formation of flagella in *rcsC11* BS#1 mutant, the swarming cell were fluorescently labeled to look for changes in number of flagellar basal structures assembled compared to the wild-type strain. We used a GFP fusion to a component of the C-ring, FliM, to analyze assembled C-rings by fluorescent microscopy. The wild-type strain displayed normal number hook-basal-bodies (HBB) per cell. Importantly, the numbers of completed

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HBBs in the rsC11 BS #1 mutant were greatly increased. This data demonstrate that the rsC11 BS #1 mutant bypass the inhibition of the flhDC in rsC11 mutant, increasing the HHB structures per cell.

In order to measured the FlgE secretion in rsC11 BS#1 mutant, we improved an established reporter system consisting of the flagellar T3S-specific substrate FlgE fused to β-lactamase lacking its own Sec-dependent secretion signal (FlgE-Bla) (12,13). The FlgE-Bla protein fusion was assayed in an flgBC mutant that results in FlgE-Bla secretion into the periplasm where it confers resistance to β-lactam antibiotics. As this fusion protein is only secreted via the flagellar-specific T3S system, this system enables us to quantify the levels of secretion of FlgE with Minimal Inhibitory Concentration (MIC) ampicillin (Ap) assays. The presence of the BS#1 point mutation in the rsC11 mutant confers resistance to high levels of Ap. The Ap’ observed in the rsC11 BS#1 mutant strain was dependent on both the flagellar gene expression and the presence of flagellar secretion apparatus.

**Conclusion**

In conclusion, we have demonstrated that the recombinant live strain constructed in this study, the rsC11 BS#1 mutant, is able to produce and express flagella. We propose that the expression of flagella in this attenuated bacterial strain will produce the delivery of these antigens to the immune system leading to a relevant immune response. Mouse immunogenicity and challenge experiments are underway to determine if this mutant can be used as oral vaccine able to confer protection against infections with a *Salmonella* virulent strain.

**References**